6. QUALITATIVE AND QUANTITATIVE ASSESSMENT OF NONCANCER HEALTH EFFECTS—DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

6.1. INTRODUCTION

Noncancer endpoints have been studied in detail in controlled laboratory animal studies of diesel exhaust, and the progression of events from initial particle deposition through chronic structural and functional alterations has been described. Some of these effects are seen early in the course of a lifetime exposure and progress throughout the lifetime of the animal in the absence of a tumor response. These findings raise the possibility of noncancer respiratory disease as a human health hazard of long-term exposure to diesel exhaust. This chapter presents a qualitative and quantitative assessment of the toxicological data on noncancer endpoints for diesel emissions.

The quantitative assessment of noncancer health effects from exposure to diesel exhaust emissions involves the development of an inhalation reference concentration (RfC). An RfC is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risks of deleterious noncancer effects during a lifetime. The RfC approach is based on the assumption that a threshold exists for the human population below which no effect will occur. The RfC is an estimate of a likely subthreshold concentration. To derive the RfC, the database on toxicological effects is reviewed and the most relevant and sensitive endpoints for human risk assessment are identified. The lowest-observed-adverse-effect level (LOAEL, the lowest concentration producing an adverse effect) or the no-observed-adverse-effect level (NOAEL, the highest concentration that did not produce any adverse effect) is used as the basis for deriving the RfC. The NOAEL (or LOAEL) for the database is selected after the human equivalent concentration is calculated for the exposure regimens used in the experimental studies. The NOAEL is considered to be an operational estimate of a subthreshold exposure. The human equivalent concentration of the NOAEL is then divided by the uncertainty factors to account for any uncertainties or data gaps necessary to extrapolate from the experimental conditions to a no-adverse-effect level in a chronically and continuously exposed sensitive human. Once consensus on the RfD derivation has been reached following both external and Agency peer review, the RfC is said to be verified and is made public through EPA's Integrated Risk Information System (IRIS).

The benchmark dose/concentration (BMC) approach may also be used to derive the RfC, as has been done for carbon disulfide, chlorodifluoromethane, and several other chemicals (U.S. EPA, 1995a). The BMC approach, applies a dose-response model to the data from key studies and then uses the dose-response relationship to interpolate an exposure concentration that is

predicted to result in a predefined response level, which is termed the benchmark response (BMR), such as a 10% incidence of a lesion or a 10% change in a continuous response variable (e.g., lung weight). The lower confidence limit on the concentration predicted to result in the BMR is the BMC, which is used as the basis for deriving the RfC. Methods for performing the BMC approach, as well as scientific consensus and Agency policy regarding the implementation of the BMC approach in risk assessment, are under development (U.S. EPA, 1995b; Barnes et al., 1995). Benchmark analyses like the one contained in this section for diesel exhaust may serve as the basis for deriving risk assessment values, as in the cases noted above, or as a point of comparison for values derived from the NOAEL/LOAEL approach.

The study or studies identifying the LOAEL, NOAEL, and/or BMC selected as the basis for deriving the RfC are termed the principal study or studies. The principal studies are those that identify the threshold region of the concentration-response curve and are representative of the entire database in this regard. Other studies that are pertinent to identifying the threshold for the effect are termed supporting studies. Supporting studies may provide additional evidence identifying the concentration-response relationship, the relative sensitivity of various effects or species, or the occurrence of other noncancer endpoints, such as reproductive or developmental toxicity. Principal and supporting studies used in deriving the RfC for diesel engine emissions are discussed in Sections 6.4 and 6.5, respectively, and the derivation of the RfC is discussed in Section 6.6.

6.2. DETERMINATION OF CRITICAL TARGET SITE

The noncarcinogenic effects of inhalation of diesel exhaust have been studied in many chronic and subchronic experiments in several laboratory animal species (Chapter 5). The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the phagocytosis of diesel particles by alveolar macrophages (AMs). These activated AMs release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of diesel particulate matter (DPM) increases, there are aggregations of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type II cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes. The neutrophils and AMs release mediators of inflammation and oxygen radicals, and particle-laden macrophages are functionally altered, resulting in decreased viability and impaired phagocytosis and clearance of particles. The latter series of events may result in pulmonary inflammatory, fibrotic, or emphysematous lesions. Studies showing these effects are described in Chapter 5. Epidemiologic studies of people exposed in various occupations in which diesel engines are used provide suggestive evidence for a respiratory effect. Although detailed information describing the pathogenesis of respiratory

effects in humans is lacking, the effects in human studies lend qualitative support to the findings in controlled animal studies.

The weight of evidence from the available toxicological data on diesel exhaust indicates with high confidence that inhalation of diesel exhaust can be a respiratory hazard, based on findings in multiple controlled laboratory animal studies in several species with suggestive evidence from human occupational studies. The endpoints of concern include biochemical, histological, and functional changes in the pulmonary and tracheobronchial regions. There is also some evidence for effects on respiratory system-related immune function. Although there is some suggestive evidence of liver and kidney changes in animals exposed to diesel exhaust, as well as some indication of neurotoxic effects at high concentrations, these data are inadequate to indicate that a hazard exists for these endpoints. Studies of other endpoints, including reproductive and developmental toxicity, in controlled animal exposures have shown no evidence of potential hazard.

6.3. APPROACH FOR DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

Twelve long-term (>1 year) laboratory animal inhalation studies of diesel engine emissions have been conducted. The focus of these studies has been on the respiratory tract effects in the pulmonary region. Effects in the upper respiratory tract and in other organs were not found consistently in chronic animal exposures. The research programs on the toxicology of diesel emissions at the Inhalation Toxicology Research Institute (ITRI) and the Japanese Health Effects Research Program (HERP) consisted of large-scale chronic exposures, with exposed animals being designated for the study of various endpoints and at various time points (Ishinishi et al., 1986, 1988; Mauderly et al., 1987a,b, 1988; Henderson et al., 1988; Wolff et al., 1987). Each research program is represented by multiple published accounts of results. These programs were selected as the principal basis for deriving the RfC because each contains studies that identify an LOAEL and an NOAEL for respiratory effects after chronic exposure (see Section 5.2) as well as pulmonary histopathology.

Diesel particulate matter is composed of an insoluble carbon core with a surface coating of relatively soluble organic constituents. Because macrophage accumulation, epithelial histopathology, and reduced clearance have been observed in rodents exposed to high concentrations of chemically inert particles (Morrow, 1992), it appears possible that the toxicity of DPM results from the carbon core rather than from the associated organics. However, the organic component of diesel particles, consisting of a large number of polycyclic aromatic hydrocarbons and heterocyclic compounds and their derivatives (Chapter 2), may also play a role in the pulmonary toxicity of DPM. It is not possible to separate the carbon core from the

adsorbed organics to compare the toxicity. Therefore, the whole particle was used as the dosimeter. See Chapters 5 and 10 for further details.

The use of a specific retention or physiologically based pharmacokinetic model is considered the optimum method for RfC derivation, and default approaches are described for chemicals without applicable models. A model developed by Yu and Yoon (1990) that accounts for species differences in deposition efficiency, normal and particle overload lung clearance rates, respiratory exchange rates, and particle transport to lung-associated lymph nodes was selected for development of the RfC. Because the dependence of mechanical alveolar clearance on particle lung burden in humans is not known, it was assumed in development of the model for humans that the particle overload phenomenon occurs in humans and in rats at equivalent lung burdens expressed as mass per unit surface area (Yu and Yoon, 1990). This assumption allows for the development of a diesel particle-specific human retention model and therefore allows extrapolation from the rat studies to human exposures. The model has not been extended to other species at this time because data describing the dependence of the particle overload phenomenon on lung particle burden for species other than the rat are not available. See Chapter 4 for further discussion of the model.

The input data required to run the dosimetric model include the particle size characterization expressed as mass median aerodynamic diameter (MMAD) and the geometric standard deviation (σg). In the principal and supporting studies used for the RfC derivation, these parameters are measured using different methods and are reported in different levels of detail. Simulation data presented by Yu and Xu (1986) show that across a range of MMAD and σg inclusive of the values reported in these studies, the pulmonary deposition fraction differs by no more than 20%. The minimal effect of even a large distribution of particle size on deposition probably results because the particles are still mostly in the submicron range and deposition is influenced primarily by diffusion. However, it has also been shown that the particle characteristics in a diesel exhaust exposure study depend very much on the procedures used to generate the chamber atmosphere. Especially important are the volume and temperature of the dilution gas, because of the rapid coagulation of particles. The differences reported in particle sizes and distributions in various studies likely reflected real differences in the exposure chambers as well as different analytical methods. Because the particle diameter and size distribution were not reported in the two lowest exposure concentrations in the HERP studies, it was decided to use a default particle size of MMAD = 0.2 μm and $\sigma g = 2.3$ for modeling of lung burden. For consistency, the lung burdens for the other studies were also calculated using the default particle size assumption. The difference in the human equivalent concentration using the default particle size compared with the actual reported particle size is no more than 4% in the HERP study and 19% in the ITRI study.

6.4. THE PRINCIPAL STUDIES FOR INHALATION REFERENCE CONCENTRATION DERIVATION

The experimental protocol and results for the principal studies are discussed in Chapter 5 and Appendix A and are briefly reviewed here. In studies conducted at ITRI, rats and mice were exposed to target DPM concentrations of 0, 0.35, 3.5, or 7 mg/m^3 for 7 h/day, 5 days/week for up to 30 mo (rats) or 24 mo (mice) (Mauderly et al., 1988). A total of 364 to 367 rats per exposure level were exposed and used for various studies examining different endpoints such as carcinogenicity, respiratory tract histopathology and morphometric analysis, particle clearance, lung burden of DPM, pulmonary function testing, lung biochemistry, lung lavage biochemistry and cytology, immune function, and lung cell labeling index. Subsets of animals were examined at 6, 12, 18, and 24 mo of exposure and surviving rats were examined at 30 mo. Diesel emissions from a 5.7-L engine operated on a Federal Test Procedure urban driving cycle were diluted and fed into the exposure chambers. Particle concentrations were measured daily using a filter sample, and weekly grab samples were taken to measure gaseous components including carbon monoxide, carbon dioxide, nitrogen oxides, ammonia, and hydrocarbons. The actual DPM concentrations for the low-, medium-, and high-exposure levels were 0.353, 3.47, and 7.08 mg/m^3, respectively. Mass median diameters (geometric standard deviations) determined using an impactor/parallel flow diffusion battery were 0.262 (4.2), 0.249 (4.5), and 0.234 (4.4) for the low-, medium-, and high-exposure groups, respectively.

Lung wet weight to dry weight ratio was increased significantly in the two highest exposure groups. Qualitative descriptions of the histological results in the respiratory tract are found in Mauderly et al. (1987a, 1988), Henderson et al. (1988), and McClellan et al. (1986). Aggregates of particle-laden AMs were seen after 6 mo in rats exposed to 7 mg/m^3 DPM target concentrations, and after 1 year of exposure histological changes were seen, including focal areas of epithelial metaplasia. Fibrosis and metaplasia increased with increasing duration of exposure and were observable in the 3.5 and 7 mg/m^3 groups of rats at 24 mo. Changes in the epithelium included extension of bronchiolar cell types into the alveoli. Focal thickening of the alveolar septa was also observed. Histological effects were seen in areas near aggregations of particle-laden AMs. The severity of inflammatory responses and fibrosis was directly related to the exposure level. In the 0.35 mg/m^3 group of rats, there was no inflammation or fibrosis. Although the mouse lungs contained higher lung burdens of DPM per gram of lung weight at each equivalent exposure concentration, there was substantially less inflammatory reaction and fibrosis than was the case in rats. Fibrosis was observed only in the lungs of mice exposed at 7 mg/m^3 DPM and consisted of fine fibrillar thickening of occasional alveolar septa.

Groups of 16 rats and mice (8/sex) were subjected to bronchoalveolar lavage after 6, 12, 18, and 24 (rats only) mo of exposure (Henderson et al., 1988). Lung wet weights were

increased at 7 mg/m^3 in mice and rats at all time points and in mice at 3.5 mg/m^3 at all time points after 6 mo. An increase in lavagable neutrophils, indicating an inflammatory response in the lung, was seen at 3.5 and 7 mg/m^3 in rats and mice at most time points. An increase in protein content of the bronchoalveolar lavage fluid was observed in rats exposed to 3.5 or 7 mg/m^3 at 12 and 18 mo but not at 24 mo. Increased protein content was also seen in mice at the two higher concentrations at all time points. Increases in lavage fluid content of lactate dehydrogenase, glutathione reductase, β-glucuronidase, glutathione, and hydroxyproline were observed in rats and mice exposed to 3.5 or 7 mg/m^3 at various time points. At the lowest exposure level, no biochemical or cytological changes occurred in the lavage fluid or in lung tissue in either Fischer 344 rats or CD-1 mice.

Mauderly et al. (1988; see also McClellan et al., 1986) examined the impairment of respiratory function in rats exposed according to the protocol described above. After 24 mo of exposure to 7 mg/m^3 DPM, mean TLC, C_{dyn}, quasi-static chord compliance, and CO diffusing capacity were significantly lower than control values, and nitrogen washout and percentage of forced vital capacity expired in 0.1 s were significantly greater than control values. There was no evidence of airflow obstruction. Similar functional alterations were observed in the rats exposed to 3.5 mg/m^3 DPM, but such changes usually occurred later in the exposure period and were generally less pronounced. There were no significant decrements in pulmonary function for the 0.35 mg/m^3 group at any time during the study.

Wolff et al. (1987) investigated alterations in particle clearance from the lungs of rats in the ITRI study. Progressive increases in lung burdens were observed over time in the 3.5 and 7.0 mg/m^3 exposure groups. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of gallium oxide (0.1 μm MMAD) as early as 6 mo at the 7.0 mg/m^3 level and 18 mo at the 3.5 mg/m^3 level; no significant changes were seen at the 0.35 mg/m^3 level. Rats that inhaled fused aluminosilicate particles (2 μm MMAD) radiolabeled with cesium after 24 mo of diesel exhaust exposure showed increased clearance half-times in the 3.5 and 7.0 mg/m^3 groups.

In the HERP studies, histopathological effects of diesel exhaust on the lungs of rats were investigated (Ishinishi et al., 1986, 1988). In this study, both light-duty (LD, 1.8-L) and heavy-duty (HD, 11-L) diesel engines were operated under constant velocity and load conditions. The exhaust was diluted to achieve target concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD only) mg/m^3 DPM. Particle concentrations were determined by filter samples. Actual concentrations were 0.11, 0.41, 1.18, and 2.32 mg/m^3 for the light-duty engine and 0.46, 0.96, 1.84, and 3.72 mg/m^3 for the heavy-duty engine. Fischer 344 rats (120 males and 95 females per exposure level for each engine type) were exposed for 16 h/day, 6 days/week for 30 mo. Particle size distributions were determined using an Andersen

cascade impactor and an electrical aerosol analyzer. At the 24-mo sampling, the MMAD and distribution (σg) were 0.22 (2.93) and 0.19 (2.71) for the light-duty engine groups at 2.32 and 1.18 mg/m^3, respectively, and 0.27 (3.18) and 0.22 (2.93) for the heavy-duty engine groups at 3.72 and 1.84 mg/m^3, respectively (Ishinishi et al., 1988). The number and timing of the samples are not clear from the published reports, nor is it clear which method was used for the results reported above. Particle size data were not reported for the other exposure groups. Hematology, clinical chemistry, urinalysis, and light and electron microscopic examinations were performed. The body weight of females exposed to 4 mg/m^3 DPM was 15% to 20% less than that of controls throughout the study. No histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m^3 DPM or less. At concentrations above 0.4 mg/m^3 DPM, accumulation of particle-laden AMs was observed. In areas of AM accumulation, there was bronchiolization of the alveolar ducts, with bronchiolar epithelium replacing alveolar epithelium. Proliferation of brochiolar epithelium and Type II cells was observed. In these areas, edematous thickening and fibrosis of the alveolar septum were seen. Fibrosis of the alveolar septum developed into small fibrotic lesions. These lesions are collectively referred to as hyperplastic lesions by the authors and their incidence is reported. From a total of 123 to 125 animals examined (approximately equal numbers of males and females), hyperplastic lesions were reported in 4, 4, 6, 12, and 87 animals in the light-duty engine groups exposed to 0, 0.11, 0.41, 1.18, and 2.32 mg/m^3 DPM, respectively, and in 1, 3, 7, 14, and 25 animals in the heavy-duty engine groups exposed to 0, 0.46, 0.96, 1.84, and 3.72 mg/m^3 DPM, respectively. Statistical analysis of these results was not reported, but there was no difference in the severity ascribed to changes in pulmonary pathology at similar exposure concentrations between the LD and the HD series.

The ITRI and HERP studies are complementary for identifying the critical effect and its LOAEL and NOAEL. The ITRI study provides results on many different endpoints reflecting pulmonary toxicity, and the effect levels are the same, but the LOAEL and NOAEL are different by a factor of 10. In the HERP study, the concentrations differ by a factor of 2-4, but only histopathology is reported. Taken together, these two studies (including several published reports for the ITRI study) provide good definition of the low-concentration effects of diesel emissions.

The HERP study identifies LOAELs for rats exposed chronically at 1.18 and 0.96 mg/m^3 (actual exposure) for the LD and HD series, respectively, and NOAELs at 0.41 and 0.46 mg/m^3 (actual) for the LD and HD series. The ITRI studies identify a NOAEL for biochemical, histological, and functional changes in the pulmonary region at 0.35 mg/m^3 (LOAEL = 3.5 mg/m^3). The human equivalent concentrations (HECs) for the principal studies were obtained using the deposition and retention model of Yu and Yoon (1990), as discussed previously. The HEC calculation is based on the assumption that the estimate for the human exposure scenario (a 70-year continuous exposure) should result in an equivalent dose metric, expressed as mass of

diesel particle carbon core per unit of pulmonary region surface area, to that associated with no effect at the end of the 2-year rat study. To obtain the HEC, the lung burden in the rat study is calculated using the exposure regimen (concentration, number of hours per day, and days per week) and values for rat tidal volume, functional residual capacity, and breathing frequency. A continuous human exposure resulting in the same final lung burden is calculated and is the HEC. The HEC values corresponding to the animals' exposure levels in the principal studies are shown in Table 6-1, along with a designation of the concentrations as AEL (adverse-effects level) or NOAEL; the LOAELs (HEC) are 0.30, 0.36, and 0.36 mg/m^3. These values, along with the LOAELs from other studies (discussed below), show strong support for an experimental threshold in rats in the range of 0.15 to 0.3 mg/m^3 DPM. The highest NOAEL (HEC), which is below all LOAELs (HEC), is 0.155 mg/m^3 DPM from the HERP heavy-duty diesel study. This NOAEL (HEC) is selected as the basis for the RfC calculation.

6.5. SUPPORTING STUDIES FOR INHALATION REFERENCE CONCENTRATION DERIVATION

Chronic inhalation studies using male F344 rats and male Hartley guinea pigs were carried out at the General Motors (GM) Research Laboratories (Barnhart et al., 1981, 1982). Exposures to target concentrations of 0.25, 0.75, and 1.5 mg/m^3 DPM were generated 20 h/day, 5.5 days/week for up to 2 years. Exposures at 0.75 and 1.5 mg/m^3 for 2 weeks to 6 mo were reported by Barnhart et al. (1981, 1982). The focus of these studies is on electron micrographic morphometry, and very little descriptive light microscopic histology is reported. These data show that no appreciable changes in morphometric parameters occurred after a 2-year exposure to 0.25 mg/m^3, while exposure to 0.75 or 1.5 mg/m^3 DPM resulted in increased thickness of alveolar septa and increased number of various types of alveolar cells. Increased numbers of

Table 6-1. Human equivalent continuous concentrations from the principal studies

Study	Exposure concentration (mg/m^3)	AEL/NOAEL[a]	HEC[b] (mg/m^3)
HERP-Light Duty	0.11	NOAEL	0.038
	0.41	NOAEL	0.139
	1.18	AEL	0.359
	2.32	AEL	0.571
HERP-Heavy Duty	0.46	NOAEL	0.155
	0.96	AEL	0.303
	1.84	AEL	0.493
	3.72	AEL	0.911
ITRI	0.353	NOAEL	0.042
	3.47	AEL	0.360
	7.08	AEL	0.582

[a]AEL: adverse-effects level; NOAEL: no-observed-adverse-effect level.
[b]HEC: human equivalent concentration.

PMNs and monocytes were lavaged from rats exposed to 0.75 or 1.5 mg/m^3, and biochemical changes occurred in lung tissue at these concentrations (Misiorowski et al., 1980; Eskelson et al., 1981; Strom, 1984). These studies demonstrate an LOAEL of 0.796 mg/m^3 DPM and a NOAEL of 0.258 mg/m^3 DPM for male guinea pigs in a chronic study for respiratory endpoints, including light and electron microscopy, lavage cytology, and lung tissue biochemistry.

A 15-mo inhalation study was performed by Southwest Research Institute for General Motors (Kaplan et al., 1983). Male F344 rats, Syrian golden hamsters, and A/J mice were exposed to diluted diesel exhaust at target concentrations of 0.25, 0.75, and 1.5 mg/m^3 for 20 h/day and 7 days/week. Focal accumulation of particle-laden AMs was associated with minimal to mild fibrosis of the alveolar wall. Based on accumulation of particle-laden macrophages, this study identifies an LOAEL at 0.735 mg/m^3 and an NOAEL at 0.242 mg/m^3.

In a study performed by NIOSH (Lewis et al., 1986, 1989; Green et al., 1983), male and female F344 rats and male Cynomolgus monkeys were exposed to target levels of 2 mg/m^3 diesel particles. Accumulations of black-pigmented alveolar macrophages were seen in the alveolar

ducts of rats adjacent to terminal bronchioles, and epithelial lining cells adjacent to collections of pigmented macrophages showed marked Type II cell hyperplasia. No evidence of impaired pulmonary function as a result of the exposure to diesel exhaust was found in rats. Histological examination of lung tissue from monkeys exposed for 24 mo in the same regimen used for rats revealed aggregates of black particles, principally in the distal airways of the lung. Fibrosis, focal emphysema, or inflammation was not observed. The monkeys exposed to diesel exhaust demonstrated small airway obstructive disease. This study demonstrates an LOAEL for rats and monkeys at a diesel particle concentration of 2 mg/m^3. Although the data suggest that the pulmonary function effect in primates more closely resembles that in humans, this study had only one exposed group, making evaluation of dose response impossible. Thus, it was not considered sufficient to eliminate consideration of the strong rodent database.

Heinrich et al. (1986; see also Stöber, 1986) exposed male and female Syrian golden hamsters, female NMRI mice, and female Wistar rats to diesel engine emissions with a 4.2 mg/m^3 particulate concentration. Lung weights were increased by a factor of 2 or 3 in rats and mice after 2 years of exposure, and in hamsters the lung weights were increased by 50% to 70%. Although histological examination revealed different levels of response among the three species, histological effects were seen in all species and effects on pulmonary function were observed in rats and hamsters. This study demonstrates an LOAEL of 4.2 mg/m^3 in rats for respiratory system effects.

The effects of diesel exhaust on the lungs of 18-week-old male Wistar rats exposed to 8.3 ± 2.0 mg/m^3 particulate matter were investigated by Karagianes et al. (1981). Histological examinations of lung tissue noted focal aggregation of particle-laden alveolar macrophages, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema. Lesion severity was related to length of exposure. No exposure-related effects were seen in the nose, larynx, or trachea. This study demonstrates an LOAEL of 8.3 mg/m^3 DPM for respiratory effects after chronic exposure of rats to diesel emissions.

Lung function was studied in adult cats chronically exposed to diesel exhaust concentrations of 6.34 mg/m^3 for the first 61 weeks and 6.7 mg/m^3 from weeks 62 to 124. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks (Pepelko et al., 1980).

Heinrich et al. (1995) exposed Wistar rats to diesel exhaust at DPM concentrations of 0.8, 2.5, and 7 mg/m^3, 18 h/day, 5 days/week for 24 mo. Body weights were significantly decreased in the two higher exposure groups. Bronchoalveolar hyperplasia and interstitial fibrosis of increasing incidence and severity at greater concentrations were seen in all exposure groups. This study demonstrates an LOAEL of 0.8 mg/m^3.

Nikula et al. (1995) exposed Fischer 344 rats to diesel exhaust at DPM concentrations of 2.4 and 6.3 mg/m^3 16 h/day, 5 days/week for 23 mo. Survival was decreased in the high-exposure males, while body weights were reduced in both males and females in the high-exposure group. Pulmonary hyperplasia, inflammation, and fibrosis were seen in a high percentage of rats in both exposure groups. The high exposure concentrations precluded use of this study for the development of an RfC.

Werchowski et al. (1980a) reported a developmental study in rabbits exposed on days 6 through 18 of gestation to a 1-in-10 dilution of diesel exhaust (DPM concentration ≈ 12 mg/m^3). Exposure to diesel emissions had no effect on maternal toxicity or on the developing fetuses. In a companion study (Werchowski et al., 1980b), 20 SD rats were exposed for 8 h/day during days 5 to 16 to a target concentration of 12 mg/m^3 of DPM. Fetuses were examined for external, internal, and skeletal malformations, and the number of live and dead fetuses, resorptions, implants, corpora lutea, fetal weight, litter weight, sex ratio, and maternal toxicity were recorded. No conclusive evidence of developmental effects was observed in this study.

In an EPA-sponsored reproductive study summarized by Pepelko and Peraino (1983), CD-1 mice were exposed to a target concentration of 12 mg/m^3 DPM for 8 h/day and 7 days/week. The F_0 and F_1 animals were exposed for 100 days prior to breeding, and 100 mating pairs were randomly assigned to four exposure groups of 25 each. Viability counts and pup weights were recorded at 4, 7, and 14 days after birth and at weaning. No treatment-related effects on body weight in F_0 mice or in F_1 animals through weaning or in mating animals through gestation were found. No treatment-related effects on gestation length, percent fertile, litter size, or pup survival were observed. The only organ weight difference was an increase in lung weight in exposed F_0 and F_1 mice (lung weight and lung weight/body weight) and in F_2 males (lung weight/body weight). Based on this study, an NOAEL for reproductive effects in rats is identified at 12 mg/m^3 DPM.

The reproductive and developmental studies described in Chapter 5 show that the effects in the respiratory system are the most sensitive effects that result from diesel exhaust exposures. These studies add to the confidence that a variety of noncancer effects have been studied and are required for a designation of high confidence in the database and the RfC.

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers. The human studies, taken together, are suggestive but inconclusive of an effect on pulmonary function, as described in Chapter 5. The studies are not directly useful for deriving the RfC because of inadequate ability to directly relate the observed effects to known concentrations of DPM. The studies are confounded by coexposures to other particles or by a lack of measurement of particle exposure.

6.5.1. Respiratory Tract Effects in Species Other Than the Rat

In several of the chronic inhalation studies described in Chapter 5, one or more species other than the rat were also exposed and examined for toxic effects. These should provide a basis for comparison of the effects in rats with the effects in other species. In the study performed at ITRI (Henderson et al., 1988; Mauderly et al., 1988), male and female CD-1 mice were exposed similarly to the rats. The LOAEL and NOAEL in rats and mice from this study would be the same, with the NOAEL for respiratory tract effects being 0.35 mg/m^3 DPM (duration-adjusted NOAEL is 0.074 mg/m^3), although some differences in the severity of the effect were apparent.

In the study conducted by the GM Biomedical Science Department (Barnhart et al., 1981, 1982; Strom, 1984; Gross, 1981), male Hartley guinea pigs as well as F344 rats were chronically exposed to 0.258, 0.796, and 1.53 mg/m^3 DPM. The evidence from this study leads to the conclusion that the LOAEL and NOAEL for rats and guinea pigs are the same, although important differences in the endpoints were reported in the two species. The NOAEL is 0.258 mg/m^3 (duration adjusted NOAEL is 0.17 mg/m^3).

Kaplan et al. (1982) reported a subchronic study in F344 rats, A/J mice, and Syrian golden hamsters exposed to 1.5 mg/m^3 DPM. The histological observations, including AM accumulation and associated thickening of the alveolar wall, were described together, with no distinction between species, suggesting that the observed effects were similar in the species examined. Kaplan et al. (1983) reported a 15-mo study in which F344 rats, A/J mice, and Syrian golden hamsters were exposed to 0.25, 0.75, or 1.5 mg/m^3 DPM. No exposure-related lesions were found in tissues other than the respiratory tract. Based on particle-laden AM accumulation, this study identifies an LOAEL at 0.735 mg/m^3 and an NOAEL at 0.242 mg/m^3. The descriptions provided suggest that the pulmonary effects were similar across the three species examined, but this conclusion is compromised by the lack of detailed reporting and the possibility of intercurrent infection in rats and poor animal health (as evidenced by poor growth) in hamsters. The duration adjusted NOAEL is 0.202 mg/m^3.

Lewis et al. (1986, 1989) exposed rats and monkeys to 2 mg/m^3 DPM for 2 years and reported pulmonary function and histopathology. Pulmonary function was affected in both species, although with a different pattern of response, as discussed in Chapter 5. Significant differences were observed in the histopathological response. In monkeys, slight particle accumulation was observed, but no fibrosis, focal emphysema, or inflammation was present. Rat lungs in this experiment showed AM accumulation, multifocal histiocytosis, and associated fibrosis and inflammatory cells in the interstitium.

Heinrich et al. (1986) exposed Wistar rats, Syrian golden hamsters, and NMRI mice chronically to 4 mg/m^3 DPM. Lung weight was increased two-fold in mice, 1.5-fold in hamsters, and three-fold in rats. The activity of enzymes recovered in bronchoalveolar lavage was increased

to roughly the same extent in rats, mice, and hamsters. Hamsters showed thickened alveolar septa and slight epithelial hyperplasia, with no AM accumulation. Mice also showed epithelial hyperplasia and interstitial fibrosis. Rat lungs had severe inflammatory changes, thickened alveolar septa, hyperplasia, and metaplasia. This study presents the clearest indication of a possibly greater severity in rats compared with other rodent species for noncancer effects. It also suggests that the effect in rats may be qualitatively different, with AM accumulation playing a greater role in pathogenesis in rats than in other rodent species.

Heinrich et al. (1995) also compared effects of chronic diesel exposure on rats and two strains of mice exposed to fairly high concentrations of diesel particles. Similar lung burdens were reported in rats and mice on the basis of particle mass per unit lung wet weight. Lung weight was increased to about the same extent in rats and mice. However, the study is focused on cancer effects, and insufficient information is provided to make a detailed comparison of noncancer histopathology in rats and mice.

Several of the studies described above and in Chapter 7 suggest a significant difference in the carcinogenic response of rats and other experimental animal species. It is less clear whether such a difference holds for noncancer effects at lower exposure levels. The studies described above show similar effect levels for different species for effects that occur earlier or at lower exposure concentration, including accumulation of particles, bronchoalveolar lavage measurements, lung weight, and minor epithelial thickening and hyperplasia. At higher diesel concentrations there are clear differences between rats and the other species tested, especially in the progression to more severe histologically observed endpoints, such as hyperplasia, metaplasia, and inflammatory response. Thus the NOAEL for chronic effects of diesel does not appear to be substantially different among species, although there is some suggestion in the literature of a more sensitive as well as qualitatively different response in rats. This comparison is weakened by the fact that the published reports often give less emphasis to noncancer responses and because the effects in rats and other species are not always measured or reported in the same way. The pathogenesis of diesel exhaust effects has not been studied as thoroughly in any other species as it has in the rat. For example, no specific measurement of particle clearance from the lung has been reported in any species other than the rat. Within the resolving power of the available studies, it is concluded that there is limited evidence for a difference in the NOAEL for noncancer effects across species, but the evidence is not adequate to quantitatively define the difference, especially at low exposure concentrations. Hence there is no clearly more appropriate species on which the RfC derivation for noncancer effects should be based.

Mice were included in the ITRI, Kaplan et al. (1982), and Heinrich et al. (1986, 1995) studies. The Heinrich studies used a single exposure to high concentrations and are supportive of the other results in mice but are not appropriate to define an LOAEL for mice. The Kaplan study

defines an LOAEL and NOAEL of 0.735 and 0.242 mg/m^3 DPM, respectively. The duration-adjusted LOAEL and NOAEL are 0.613 and 0.202 mg/m^3, respectively. The ITRI study defined the adjusted LOAEL and NOAEL at 0.723 and 0.074 mg/m^3, respectively. Because the dose spacing is so wide in the ITRI study, the Kaplan study is more appropriate for defining an NOAEL. Likewise, the Kaplan et al. study is the only multiple-dose study in hamsters, and it defines the same LOAEL and NOAEL for hamsters as for mice. The GM study is the only chronic study in guinea pigs, and it defines the LOAEL and NOAEL for this species at 0.796 and 0.258 mg/m^3, respectively. The adjusted LOAEL and NOAEL for guinea pigs from the GM study are 0.52 and 0.17 mg/m^3, respectively. The effects levels for mice, hamsters, and guinea pigs are similar to the duration-adjusted LOAEL and NOAEL for rats, which are 0.723 mg/m^3 (from ITRI study) and 0.26 mg/m^3 (from Ishinishi et al., 1988), respectively. If the RfC were to be derived based on the duration-adjusted NOAEL, the rat data would be preferred because of the more complete database of chronic rat studies and the more complete presentation of the noncancer endpoints in the rat studies.

The method for deriving inhalation RfCs (U.S. EPA, 1994) includes dosimetric adjustments of animal exposure to arrive at a human equivalent concentration. The default calculation of an HEC for a particle exposure uses the ratio of animal to human regional deposited dose (RDDR) to a specific region of the respiratory tract. The methods also allow replacement of the default approach when a better model is available. The derivation of the RfC in this case makes use of the Yu and Yoon (1990) model to calculate the HEC from the rat studies. Since the Yu and Yoon model has been developed only for the rat-to-human extrapolation, the chosen approach assumes that dosimetric differences between rats and other small animal species would not result in a substantially lower HEC. The LOAEL (HEC) and NOAEL (HEC) from the rat studies based on the Yu and Yoon model are 0.36 and 0.155 mg/m^3, respectively.

6.6. DERIVATION OF THE INHALATION REFERENCE CONCENTRATION

Studies of chronic exposures to diesel emissions performed at ITRI and HERP were selected as the basis of the RfC because they identify both an NOAEL and an LOAEL for rats exposed chronically. The only other study identifying both an NOAEL and an LOAEL was the GM study, which was not used because information characterizing the pulmonary lesions in rats was limited. The availability of the dosimetric model for rats and not for other species, along with the apparent comparability between the rat and other rodent species in response, resulted in choosing the rat as the basis for developing the RfC. Although the data from the monkey in the Lewis et al. (1989) study suggest that the pulmonary function effect in primates more closely resembles that in humans, this study had only one exposed group, making evaluation of dose response impossible. Thus, this was not considered to be a strong enough basis to eliminate

consideration of the strong rodent database. The pulmonary effects, including histological lesions, biochemical changes, pulmonary function impairment, and impaired particle clearance, were determined to be the critical noncancer effect. Sufficient documentation from other studies showed that there is no effect in the extrathoracic region of the respiratory system or in other organs at the lowest levels that produce pulmonary effects in chronic exposures. In addition, adequate information is available from the EPA studies showing no effect on development in two species or on reproduction in a two-generation reproductive study.

Because the RfC is based on an NOAEL from a chronic animal study, uncertainty exists in the extrapolation from animals to humans and for extrapolation to sensitive members of the population (inter- and intraspecies extrapolation). A default factor of 10 is normally applied for each area of uncertainty (i.e., a total uncertainty factor of 100) when a chronic animal NOAEL is available. Since a dosimetry model specifically for diesel particles is available, the use of this model is considered to reduce the uncertainty in extrapolating between animals and humans, compared to a case in which no chemical or species-specific data on dosimetry are available. The default uncertainty factor of 10 includes aspects of pharmacokinetics and pharmacodynamics. An uncertainty factor of 1 rather than 10 was adopted for interspecies extrapolation and was used for the diesel RfC. The uncertainty factor for interspecies extrapolation is normally reduced when kinetic data are available, as in this case, which reduces the uncertainty in extrapolating from animals to humans by accounting for the kinetic differences with data. A further reduction was considered appropriate, as recommended by peer reviewers, on the basis that substantial information suggests that the rat may be a very sensitive species, compared with humans, to the effects of inhalation of diesel particles. There is some evidence, as discussed above, that rats may be more sensitive than other rodent species. There is also evidence from the Lewis et al. (1989) study that rats may be more sensitive than monkeys. Humans would be expected to be more similar to monkeys because their respiratory tract structure is more similar and because of their closer phylogenetic relationship. In the comparison of rat effects with those on other rodents and monkeys, there is also limited evidence that the responses are qualitatively different, with a much greater role for macrophage dysfunction and accumulation and associated epithelial effects in rats. These lines of evidence, which are fairly limited individually, lead to a reasonably strong argument for a reduction in the uncertainty factor when considered together. A total uncertainty factor of 10 results for intraspecies extrapolation.

With the NOAEL (HEC) of 0.155 mg/m^3 DPM from the HERP study, an RfC of 16 $\mu g/m^3$ was calculated. The RfC also includes confidence statements associated with the principal study, the database, and the resulting RfC. The studies used as the basis for the RfC were well-conducted chronic studies with adequate numbers of animals, and the LOAELs and NOAELs were consistent across studies, thereby resulting in high confidence. The database contains

several chronic studies, including multiple species, that support the LOAEL observed in the principal studies. There are also developmental and reproductive studies, resulting in a high-confidence data base. Because of the high confidence in the studies and database, the RfC has high confidence.

6.6.1. Application of the Benchmark Dose Approach to Derivation of the RfC

An alternative to deriving the RfC based on the NOAEL identified in the animal studies is application of the benchmark dose/concentration approach. The BMC was described by Crump (1984) and recently discussed by EPA (1995b). The BMC approach involves fitting a dose-response function to dose and effect information from a single study and using the dose-response curve to predict the dose that will result in a level of response that is defined a priori as the benchmark response. For example, a 10% increase in incidence of epithelial hyperplasia might be defined as the benchmark response, and a dose-response curve relating inhaled DPM to hyperplasia in rats exposed chronically to diesel exhaust would be used to estimate the exposure concentration resulting in a 10% increase. The lower confidence limit of that concentration is the BMC, and it is used as the representative value for the dose-response assessment.

For diesel exhaust there are several chronic exposure studies in animals that could be used to estimate a benchmark concentration. Software for performing benchmark dose calculations is available commercially, and benchmark dose programs also can be developed using standard statistical software. Different approaches are used for modeling dichotomous versus continuous data. Dichotomous models estimate the probability of the effect being modeled at a given dose, and continuous models estimate the magnitude of the response at a given dose. This fundamentally different output causes difficulty in cases such as the database for diesel exhaust that include both types of data, because comparisons of the two types of results must be made to select the most appropriate BMC for the dose-response assessment. Crump (1995) has developed an approach to modeling continuous data that models dose against the distribution of the continuous variable and estimates the probability of an abnormal response at a given dose using a preselected magnitude of response to define an abnormal response. This approach has the distinct advantage of expressing the results for dichotomous and continuous data in the same terms, but it has not been evaluated extensively and is not readily available.

A first step in carrying out a BMC analysis is selecting studies and data sets that are appropriate to model. Minimum data criteria have not been clearly established. As provisional criteria, the analyses that follow will require that complete information on the response of interest should be available and that at least two exposure levels with responses that differ from those of the controls are needed. Based on this criterion, studies with a single exposure concentration

(including Lewis et al., 1989; Heinrich et al., 1986; Iwai et al., 1986; Karagianes et al., 1981; Pepelko et al., 1980; and the mouse data from Heinrich et al., 1995) are not amenable to the BMC approach. In addition, the rat data from Heinrich et al. (1995) and Creutzenberg et al. (1990) are not amenable to BMC analysis because the information on noncancer histopathology is not reported in detail and the information on lung clearance rates is reported as group means with no standard deviation.

To perform a dose-response analysis for a continuous variable, either a measure of variability or the individual animal measurements are needed. The study reported by Kaplan et al. (1983) is also not considered useful for BMC analysis. The principal result of interest in the Kaplan study is reported as the incidence of pneumoconiosis in rats, mice, and hamsters. The term pneumoconiosis appears to be applied to the presence of DPM in the lung and in AMs as well as AM accumulation and any secondary effects in the epithelium. Epithelial effects in the area near particle-laden AMs are discussed in the text of the Kaplan study but are not listed as a separate entity in the tables of effects. Thus the effect termed pneumoconiosis appears to include both the expected dose-related increase in particles in the lung and any adverse effects occurring secondary to particle deposition, and therefore does not represent a clearly adverse effect. The existence of some studies for which the BMC approach is feasible and some for which it is not feasible introduces a potentially serious difficulty in deciding on the most appropriate dose-response value to use in deriving the RfC. In the case of diesel exhaust, however, this concern is reduced because the studies identified as the most appropriate for RfC derivation based on NOAEL/LOAEL levels are also amenable to BMC analysis.

The studies for which BMC analyses were performed were the ITRI, General Motors, Ishinishi (1986), and Nikula et al. (1995) studies. These studies contained a variety of endpoints that could be modeled using the BMC approach. A total of 41 data sets were selected from these studies for BMC determination. Because a variety of models are available, and each model can be varied (for example, by including a background term or a threshold term), the number of model runs for a database as complete as that for diesel exhaust can become unmanageable. In addition to this large number of endpoints and models, several dose or exposure terms could be used (e.g., for rats one could use the exposure concentration, the exposure concentration × time, the duration-average exposure concentration, the lung burden estimated by a deposition model, or the human equivalent concentration based on a deposition and clearance model). Clearly, the number of possible model runs expands geometrically, and some decisions must be made to limit and focus the extent of the analyses.

In these analyses, the dose metric used for rat data was the human equivalent continuous exposure concentration based on the Yu and Yoon (1990) deposition/clearance model. For mice, the duration-averaged exposure concentration was used. The 41 data sets were modeled using

the polynomial model dichotomous or continuous data (Howe, 1990a, 1990b), based on extra risk, and including or excluding a threshold term. The inclusion of a threshold term improved model fit substantially only in cases with low-dose groups showing no response over controls. In most cases a background term was also estimated, and in some analyses with dichotomous data it was omitted. As expected, the inclusion of a background term improved model fit for dichotomous data only when there was a nonzero response in the controls. Based on the results using the polynomial model, 12 data sets were selected for analysis with the Weibull model (Howe, 1990c, 1990d) to determine the sensitivity of the BMC estimate to the choice of model. The availability of different dose metrics and different models for different endpoints introduces additional difficulty in determining the most appropriate BMC for deriving the RfC. This difficulty is lessened by the fact that the rat data for BMC analysis are the most extensive, they include several studies for which both LOAEL and NOAEL are identified, and they are also the data for which the deposition and clearance model is available. As discussed previously, the data from other species do not suggest large differences in species sensitivity at low concentrations.

Perhaps the most critical decision in the BMC approach is the level of response defined as the benchmark response. As discussed by EPA (1995b), there is an emerging consensus that a BMR of 0.05 or 0.1 is probably appropriate for dichotomous data for most endpoints. There is no clear consensus about the appropriate choice of BMR or on how to select a BMR for a continuous effect. This dilemma is the main reason for the appeal of models such as that presented by Crump (1995) or by Gaylor and Slikker (1990), which derive a BMC in terms of a probability statement. However, both of those models require that some magnitude of response be selected to delineate between “responders” and “nonresponders,” so the issue remains as to how one can consistently define a response level for the myriad endpoints found in the toxicological literature. One approach is to work backward from the way the BMC will be used

to derive the RfC. The discussion by EPA (1995b) and the precedents for the use of BMC in derivation of RfCs that are now on IRIS suggest that the BMC will be used like an NOAEL has been used in the past. It follows that the BMR should be set at a level that would not be considered adverse for the effect in question, or for an effect that is very mildly adverse such that the use of the lower confidence limit on dose results in a BMC that is in the nonadverse range under the conditions of the experiment. This provides general guidance for selecting BMRs for continuous endpoints, although the issue of maintaining consistency between endpoints remains an extremely important one because of the interrelatedness of different toxicological endpoints. The benchmark responses used in the BMC analyses for diesel exhaust are shown in Table 6-2.

It should be noted that the lack of clear policy on selecting the BMC and the lack of specific guidance for comparing different respiratory tract effects in the form of target organ-specific guidelines, along with the other issues raised above, make BMC analysis a possible but not automatic alternative to the derivation of the RfC as presented. The BMC analysis for diesel is presented here for comparison with the RfC based on the LOAEL/NOAEL from the rat studies as presented above.

In the ITRI study, in particular, many endpoints were amenable to benchmark modeling. Henderson et al. (1988) present data on lung weight and lavage biochemistry and cytology from rats and mice sacrificed at various time points during a chronic study. Wolff et al. (1987) present data on particle clearance half-times in rats. The results shown in Table 6-3 are based on the best-fitting exponential polynomial model using the BMR from Table 6-2 applied to the ITRI data (Henderson et al., 1988; Wolff et al., 1987).

Table 6-2. Definition of benchmark response levels for endpoints important in the diesel exhaust BMC analysis

Endpoint	Benchmark definition	Extra risk
Lung weight	10% increase	0.10
Bronchoalveolar lavage-number of macrophages	50% increase	0.50
Bronchoalveolar lavage-number of neutrophils	200% increase (3 × control)	2.0
Bronchoalveolar lavage-protein	100% increase (2 × control)	1.0
Bronchoalveolar lavage-enzymes	100% increase (2 × control)	1.0
Clearance half-time	20% increase	0.20
Body burden of a particle after 200 days	20% increase	0.20
Incidence of hyperplasia	10% incidence	0.10
Alveolar-capillary thickness	20% increase	0.20

Table 6-3. Results of benchmark concentration analyses using the polynomial model and data from the ITRI study

Data set, model	Benchmark response	MLE	BMC
Rats			
Male rat lung weight @ 24 mo Polynomial, no threshold	10	0.10	0.08
Female rat lung weight @ 24 mo Polynomial, no threshold	10	0.061	0.05
Rat BAL macrophages @ 24 mo, M and F combined Polynomial, no threshold	50	0.315	0.27
Rat BAL neutrophils @ 24 mo, M and F combined Polynomial, threshold	200	0.0942	0.06
Rat BAL protein @ 18 mo, M and F combined Polynomial, threshold	100	0.309	0.18
Rat BAL LDH @ 18 mo, M and F combined Polynomial, no threshold	100	0.221	0.15
Rat BAL β-glucuronidase @ 24 mo, M and F combined Polynomial, threshold	100	0.172	0.05
Rat Ga_2O_3 clearance half-time @ 6 mo, M and F combined Polynomial, no threshold	20	0.262	0.15
Rat Ga_2O_3 clearance half-time @ 12 mo, M and F combined Polynomial, no threshold	20	0.168	0.10
Rat Ga_2O_3 clearance half-time @ 24 mo, M and F combined Polynomial, no threshold	20	0.074	0.04
Rat Cs-FAP clearance half-time @ 24 mo, M and F combined Polynomial, no threshold	20	0.048	0.04
Rat Cs-FAP clearance, % of initial body burden after 200 days Polynomial, no threshold	20	0.044	0.03
Mice			
Male mouse lung weight @ 24 mo Polynomial, no threshold	10	0.13	0.11
Female mouse lung weight @ 24 mo Polynomial, no threshold	10	0.16	0.13
Mouse BAL macrophage @ 24 mo, M and F combined Polynomial, no threshold	10	Not able to converge	
Mouse BAL neutrophils @ 24 mo, M and F combined Polynomial, no threshold	200	0.640	0.47
Mouse BAL protein @ 18 mo, M and F combined Polynomial, no threshold	100	0.353	0.27
Mouse BAL LDH @ 18 mo, M and F combined Polynomial, no threshold	100	0.455	0.36
Mouse BAL β-glucuronidase @ 24 mo, M and F combined Polynomial, threshold	100	0.112	0.11

Source: Henderson et al. (1988) and Wolff et al. (1987).

The BMC values are the lower 95% confidence limit on the exposure concentration predicted to result in the BMR response level. All of the responses modeled above were data presented as continuous variables, and the model was used to estimate the exposure predicted to result in a predefined response magnitude, the BMR. Without any other adjustment for the severity of the effect, it is implicit in the BMC approach for continuous data that BMRs are assumed to represent effects of equivalent severity. Clearly, such a comparison is very subjective and cannot be made precisely. Nevertheless, the judgment of the appropriate BMR is fundamental to the application of the BMC approach.

The BMCs from the ITRI rat data were calculated using the human equivalent concentration of the rat exposures based on the Yu and Yoon (1990) dosimetry model. The NOAEL from the ITRI study was 0.35 mg/m^3 (duration-adjusted NOAEL is 0.074 mg/m^3) and the human equivalent NOAEL is 0.042 mg/m^3. This NOAEL (HEC) is lower than the NOAEL from the Ishinishi et al. study, which was used to derive the RfC because the NOAEL and LOAEL from the ITRI study differed by a factor of 10. The rat NOAEL used to derive the RfC was a NOAEL (HEC) of 0.155 mg/m^3. The BMC values based on rat lung weight were below the NOAEL from Ishinishi et al. (1986), suggesting that the lung weight is a sensitive effect. The sensitivity of the lung weight is consistent with other chronic rat studies as well as those in mice (Heinrich et al., 1995). The bronchoalveolar lavage indicators showed large variability in BMC value, with macrophages, protein, and LDH having BMCs above the range of the rat NOAEL. Lavagable neutrophils and β-glucuronidase, however, were quite sensitive. Measures of particle clearance in rat lungs were also sensitive, resulting in a range of BMC values between 0.033 and 0.146 mg/m^3. It was noted that there are no BMC estimates based on histopathological effects, even though these effects tend to be sensitive indicators of diesel exposure, because these effects were not reported in an adequately quantitative manner in the various publications describing the ITRI study.

The BMC levels based on mouse data from the ITRI study also showed a wide range, with lung weight and lavage β-glucuronidase being the most sensitive. The mouse BMCs were calculated using the duration-averaged exposure concentration as the dose term, so the appropriate NOAEL for comparison is the duration-adjusted NOAEL from the ITRI study, which was 0.074 mg/m^3. The duration-averaged LOAEL for the same study is 0.723 mg/m^3. All mouse BMC values from the ITRI study fall between the NOAEL (HEC) and the LOAEL (HEC).

The basis for the RfC was the NOAEL (HEC) in the Ishinishi study, which was 0.155 mg/m^3. This NOAEL (HEC) was selected because it was lower than the valid LOAELs in rats from all studies considered and higher than the other NOAEL (HEC) values. The only data in the Ishinishi et al. (1988) study that were amenable to BMC analysis were the incidence of hyperplastic lesions in male, female, and combined. These results are shown in Table 6-4.

The BMC for the most sensitive effect is very similar to the NOAEL (HEC). Most of the BMC values fall between the LOAEL (HEC) and the NOAEL (HEC) from the same data set. Two of the BMCs for the heavy-duty diesel experiment exceed the LOAEL (HEC). This could result from the large number of animals and close dose spacing, which allows identification of a lower LOAEL (HEC). These data sets were characterized by very low incidence of lesions at the lowest exposure level, and the assignment of the LOAEL (HEC) was difficult because of the low incidence and the lack of detailed description of the extent or severity of the response. The BMC procedure makes determination of the effect level more objective for these data.

Two other studies contained information that was amenable to the BMC approach: the chronic study done at GM and the Nikula et al. (1995) study. Results of these analyses are shown in Table 6-5.

Table 6-4. Results of benchmark concentration analyses using the polynomial model and data from the HERP study

Data set, model	Benchmark response	MLE[a]	BMC[a]
HERP light-duty diesel, hyperplastic lesions, male and female combined Polynomial, threshold, background	10	0.39	0.34
HERP light-duty diesel, hyperplastic lesions, male rats Polynomial, no threshold, background	10	0.35	0.32
HERP light-duty diesel, hyperplastic lesions, female rats Polynomial, no threshold	10	0.24	0.19
HERP heavy-duty diesel, hyperplastic lesions, male and female combined Polynomial, no threshold	10	0.49	0.38
HERP heavy-duty diesel, hyperplastic lesions, male rats Polynomial, no threshold	10	0.67	0.46
HERP heavy-duty diesel, hyperplastic lesions, female rats Polynomial, no threshold	10	0.43	0.30

[a]mg/m^3.
Source: Ishinishi et al. (1988).

Table 6-5. Results of benchmark concentration analyses using the polynomial model and data from the GM and Nikula studies

Data set, model	Benchmark response	MLE[a]	BMC[a]
GM study—continuous variables			
Male rat alveolar-capillary thickness @ 6 mo Polynomial, threshold	20	0.069	0.06
Male rat BAL PMNs @ 48 weeks Polynomial, no threshold	200	0.087	0.08
Male rat BAL macrophages @ 48 weeks Polynomial, no threshold	50	0.244	0.12
Nikula study—continuous variables			
Male RT Lu wt. @ 23 mo Polynomial, no threshold	10	0.088	0.06
Female RT Lu wt. @ 23 mo Polynomial, no threshold	10	0.039	0.03
Male RT Lu wt. @ 18 mo Polynomial, no threshold	10	0.170	0.13
Female RT Lu wt. @ 18 mo Polynomial, no threshold	10	0.122	0.04
Nikula study—dichotomous variables			
Male RT chronic inflammation @ >18 mo Polynomial, no threshold	10	0.232	0.14
Female RT chronic inflammation @ >18 mo Polynomial, no threshold	10	0.095	0.08
Female RT alveolar proteinosis @ >18 mo Polynomial, no threshold	10	0.122	0.03
Male RT bronchoalveolar metaplasia @ >18 mo Polynomial, no threshold	10	0.195	0.12
Female RT bronchoalveolar metaplasia @ >18 mo Polynomial, no threshold	10	0.026	0.02
Male RT focal fibrosis and epithelial hyperplasia @ >18 mo Polynomial, no threshold	10	0.721	0.46
Female RT focal fibrosis and epithelial hyperplasia @ >18 mo Polynomial, no threshold	10	0.336	0.20

[a]mg/m^3.
Source: Barnhart et al. (1981, 1982) and Nikula (1995).

Overall, the benchmark concentrations from several experiments and a variety of endpoints support the NOAEL and LOAEL identified from the rat database for chronic diesel exposure studies. It is common practice to apply several models to the key data sets in a benchmark concentration analysis to determine whether the BMC is significantly model dependent, and if so, to select the most appropriate model. One reason for using a BMR that is close to the observable range is that the various models used for dose-response analysis tend to diverge more as they are extrapolated to lower doses and at the BMR such extrapolation should not be necessary, so substantial model dependence of the result is less likely. Several of the data sets that resulted in lower BMC estimates were modeled using the Weibull model, and the results are shown in Table 6-6.

These results indicate that there is relatively little difference among models for a variety of studies and endpoints. The BMC analyses shown in Table 6-6 were selected because they resulted in the lowest BMC levels of the studies and endpoints evaluated. It has been suggested that the lowest BMC level should be used to derive the RfC, analogous to the use of the lowest LOAEL to derive the RfC. Selection of the most appropriate BMC to derive the RfC is complicated by the fact that there may be many BMCs from a given experiment that identifies a single LOAEL and NOAEL. The ITRI study, for example, identifies an LOAEL of 3.5 mg/m^3 and an NOAEL of 0.35 mg/m^3 (exposure concentrations), but because of the large number of endpoints studied, many BMC values are available. On the other hand, a study such as Ishinishi et al. (1986) has only one noncancer endpoint that can be used for BMC analysis, and the Heinrich et al. (1995) study had no noncancer endpoints that were presented in a sufficiently quantitative manner for BMC analysis. If these studies are not considered in an analysis because BMC models cannot be applied, substantial uncertainty would be introduced because significant information is being ignored. In addition, several caveats must be considered relating to the studies for which BMCs were calculated. The endpoints of chronic inflammation and alveolar proteinosis in the female rats in the Nikula study were based on BMC models fit to two exposure groups, and the incidences in the lowest exposed group were 49% and 83%, respectively. Thus, the BMC is estimated at a concentration well below the lowest data point, and this extrapolation may be cause for concern. This concern applies equally to the lung weight data in the Nikula study, which show a greater than twofold increase in the low-concentration females. The data on pulmonary particle clearance from the ITRI study were reported as an estimate of the clearance half-time with no measure of variability. To run the BMC model, a coefficient of variability of 20% was assumed. Thus the data themselves do not meet the minimum requirements for application of the BMC approach as discussed above. These data sets result in the lowest BMC levels in the diesel database. For several other endpoints, such as alveolar-capillary thickness

Table 6-6. Comparison of benchmark concentration calculated using the polynomial and Weibull models for selected endpoints

Data set, model	Benchmark response	MLE[a]	BMC[a]
Dichotomous variables			
HERP light-duty diesel, hyperplastic lesions, female rats			
Polynomial, no threshold	10	0.247	0.19
Weibull, no threshold	10	0.350	0.29
HERP heavy-duty diesel, hyperplastic lesions, female rats			
Polynomial, no threshold	10	0.425	0.30
Weibull, no threshold	10	0.273	0.17
Nikula study, female RT chronic inflammation @ >18 mo			
Polynomial, no threshold	10	0.095	0.08
Weibull, no threshold	10	0.102	0.09
Nikula study, female RT alveolar proteinosis @ >18 mo			
Polynomial, no threshold	10	0.122	0.03
Weibull, no threshold	10	0.175	0.03
Continuous variables			
ITRI rat Ga_2O_3 clearance half-time @ 24 mo, M and F combined			
Polynomial, no threshold	20	0.074	0.04
Weibull, threshold	20	0.176	0.07
ITRI rat Cs-FAP clearance half-time @ 24 mo, M and F combined			
Polynomial, no threshold	20	0.048	0.03
Weibull, no threshold	20	0.050	0.03
ITRI male rat lung weight @ 24 mo			
Polynomial, no threshold	10	0.103	0.08
Weibull, no threshold	10	0.103	0.08
ITRI female rat lung weight @ 24 mo			
Polynomial, no threshold	10	0.061	0.05
Weibull, no threshold	10	0.068	0.05
ITRI rat BAL neutrophils @ 24 mo, M and F combined			
Polynomial, threshold	200	0.094	0.06
Weibull, no threshold	200	0.094	0.06
ITRI rat BAL β-glucuronidase @ 24 mo, M and F combined			
Polynomial, threshold	100	0.172	0.05
Weibull, threshold	100	0.176	0.07
G.M. study, male rat alveolar-capillary thickness @ 6 mo			
Polynomial, threshold	20	0.069	0.06
Weibull, no threshold	20	0.069	0.06
G.M. study, male rat BAL PMNs @ 48 weeks			
Polynomial, no threshold	200	0.087	0.08
Weibull, no threshold	200	0.087	0.09

[a]mg/m^3.

and lavage β-glucuronidase level, it is not clear that the BMR selected (20% and 100% increase, respectively) is the appropriate level in the context of applying the BMC to derive the RfC. Despite these caveats, it is clear that the BMC values for the more sensitive endpoints tend toward concentrations that are at or below the NOAEL identified by the combined rat database. This may well result in part because the ITRI study, which is the best documented study, has a tenfold difference between the LOAEL and NOAEL, and the Ishinishi study from which the rat NOAEL was obtained uses much closer dose spacing (a factor of 2.5 between LOAEL and NOAEL) and reports in much less detail on the noncancer effects. Thus the lack of detailed investigation in the Ishinishi study might allow a higher NOAEL to be identified at a level only about one-half of the LOAELs from the ITRI, GM, Lewis et al. (1989), and Heinrich (1995) studies. In other words, the BMC analyses suggest that a lower rat NOAEL might have been identified if different dose spacing and more detailed investigation of noncancer endpoints had been used in the existing studies.

Several limitations have been mentioned in the preceding text regarding the use of the BMC analysis for deriving an RfC. The principal limitations are the following:

- Some key studies in rats have inadequate quantitative data for BMC.
- Some endpoints are amenable to BMC and others are not.
- The policy for selecting BMC from many endpoints is not yet clear.
- It is not clear how to compare dichotomous and continuous BMCs.
- There is a lack of precedent or guidance for selecting BMR levels.
- A deposition model is available only for rats (it is not clear how to compare BMCs based on deposition/retention models with BMC based on default duration-adjusted concentrations).

Because of the issues and questions raised by these aspects of the BMC approach, the BMC will not be used to derive the RfC at this time.

6.7. SUMMARY

A large number of studies of chronic DPM inhalation in laboratory animals are available. These studies characterize the respiratory effects and the concentration-response relationship of those effects in detail. Many epidemiologic studies of occupationally exposed humans also are available. The epidemiologic studies provide qualitative evidence that supports the identification of a hazard to the respiratory system from laboratory animal studies. The human studies are of limited value quantitatively because of their inadequate exposure characterization and confounding by concurrent exposure to other pollutants. The laboratory animal studies are used to derive an RfC. The chronic studies from ITRI and HERP were selected as the principal studies for RfC development. The deposition and retention model discussed in Chapter 4 and Appendix

B was used to calculate human equivalent concentrations and identified a NOAEL (HEC) of 0.155 mg/m^3 from the HERP studies and a LOAEL(HEC) of 0.36 mg/m^3 from the ITRI studies. An uncertainty factor of 10 was applied to account for sensitive members of the population, resulting in an RfC of 16 µg/m^3. The RfC is considered to have high confidence attributable to high confidence in the study and database.

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